LOW-FREQUENCY ULTRASONIC INACTIVATION OF LACTIC ACID AND AEROBIC BACTERIA IN PROCESSED MEAT

Zbigniew J.Dolatowski, Dariusz M.Stasiak

Agricultural University, Department of Animal Food Technology, ul.Doświadczalna 44, 20-236 Lublin, Poland

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Background

Meat is highly perishable food product because of its high sensitivity to microbial spoilage. The adequate meat processing, cooking, storage procedures are essential in reducing bacterial growth, increasing shelf-life potential, quality and safety of meats. Then, during last years, many scientists are testing new, alternative food technologies, such as ultrasonication. They applied ultrasound waves for homogenization, cleaning, extraction, and emulsification, and on the other hand, for non-destructive testing, physico-chemical properties estimation and many others (Dolatowski, 1999; Sajas and Gorbatow, 1978; Scherba et al., 1991). The main active force of ultrasound is mechanical in its nature, resulting in cavitation and in "micromassaging" (energy and mass transport) of meat. Especially formation and implosion of bubbles in the liquid (cavitation) generate high-energy impact waves, electrical and chemical phenomena, heating and others. Microorganisms can be inactivated. Villamiel and de Jong (2000) treated milk with low-frequency (20 kHz) ultrasound. *Pseudomonas fluorescens* and *Streptococcus thermophilus* were inactivated, although the effectiveness of treatment can be decreased by temperature increase. The inactivation of *Escherichia coli* (Hua and Thompson, 2000) depends moderately on total power, power intensity and frequency. Inactivation occurs most readily at the highest sound intensity and lower frequency. The effect of frequency and power density on the ultrasonically-enhanced killing of biofilm-sequestered *Escherichia coli* are investigated by Peterson and Pitt (2000). They conclude that low-intensity ultrasound significantly enhanced killing of biofilm *E.coli* by gentamicin. This enhancement increased with increasing of ultrasonic intensity and decreased with frequency increase. Cicciolini et al. (1997) studied the combined effect of low frequency ultrasound (20 kHz) with temperature on the survival of a strain of *Saccharomyces cerevisiae* suspended in water. The results proved that ultrasound does not destroy the cells of yeast. The damage of *S.cerevisiae* increases their sensitivity to heat. But the total effectiveness of ultrasound in killing mocroorganisms is still unclear.

Objective

The aim of the research was to evaluate the effects of low-frequency ultrasonication on microbial contamination level of ultrasonically processed meat. Another purpose was to estimate the microbial growth in cooked-ham (prepared from ultrasonically treated meat) during its chilling storage.

Methods

The meat samples were prepared post-rigor from carcasses (5 pig and 5 beef). Meat (*m.semimembranosus)* was cured (injection 15%) with typical brines (sodium chloride, nitrite) and massaged with ultrasound using experimental massaging machine. The primary, pre-rigor (2 hours after slaughter) exposition of beef samples (about 1 kg each) was equal to 2 minutes. Control samples were not ultrasonically treated. After next 24-hour chilling (7°C inside temperature), the meat was cured and mechanically massaged for 1.5 hour at 25 rpm. The further processing (smoking, cooking, storage) of beef samples was prepared according to the usual norm. Samples of pig meat were prepared from chilled (24 h, 7°C inside temperature) halves, cured and massaged for 90 min (control sample) or 60 min and 10 min with ultrasound, stored for 24 hours at 2-4°C and second time massaged (such as above). The further processing of meat samples (smoking, cooking, storage) was prepared according to the usual norm. The products were cold stored at 4-6°C.

Microbiological determinations of meat (product) were done on samples (each 20 g) diluted in 180 cm^3 physiological salt solution, plated on agar (aerobic bacteria) or Kipler's medium (lactic acid bacteria) and incubated.

Ultrasonication was realized with experimental massaging machine with 25 kHz transducers and power intensity about 2.5 $W\cdot cm^{-2}$. The processed meat temperature was measured.

The data was analysed and tested with procedures of Statgraphics v.5 package (variance analysis, t-Student test of mean).

Results and discussion

The collected data shows statistically significant ($p<0.05$) influence of low-frequency ultrasound on microbial contamination level of pig meat surface. The total number of aerobic mesophilic bacteria before meat processing was about $6\cdot 10^5$/g. After curing it decreased to about $5.5\cdot 10^4$/g. Meat massaging without ultrasound did not chang (no statistically significant changes) microbial contamination of meat – the number of aerobic bacteria after 2nd massaging was equal to $4.7\cdot 10^4$. Massaging supported with ultrasound lowered aerobic bacteria population to $1.5\cdot 10^3$/g (Fig.1).

The number of lactic acid bacteria in pig meat (before processing) was equal to $3\cdot 10^3$/g. Further processing (curing and massaging) increases it to about $7.1\cdot 10^3$/g (after the 3rd massaging). Ultrasound treatment drastically ($p<0,05$) decreases the number of lactic acid bacteria to $1.7\cdot 10^2$/g after the 1st and to $1.8\cdot 10^2$/g after the 2nd massaging (Fig.1).

The aerobic bacteria cell concentrations achieved were around $5.1\cdot 10^5$/g in pre-rigor beef (after ultrasonication). After curing, the bacterial counts were $5\cdot 10^4$/g. The growth of bacteria up to $5.6\cdot 10^5$ was confirmed after typical beef massaging. Pre-rigor ultrasound treatment of meat lowered the number of bacteria by 10-fold. Further processing of ultrasonically treated beef caused growing of aerobic bacteria to $4.3\cdot 10^5$/g, and the difference between control and ultrasonicated sample was decayed (Fig.2).

The number of lactic acid bacteria in pre-rigor beef was $4.0\cdot 10^4$ before and $1.2\cdot 10^4$ after low-frequency ultrasonic treatment. After beef curing and massaging, the cell concentration was similar in both types of samples, i.e. control – $5.1\cdot 10^5$ and ultrasonicated – $4.0\cdot 10^5$ (Fig.2). Pre-rigor beef ultrasonic treatment decreases the number of aerobic and lactic acid bacteria (statistically significant influence) but the effect of sonication was decayed in further processing of meat.

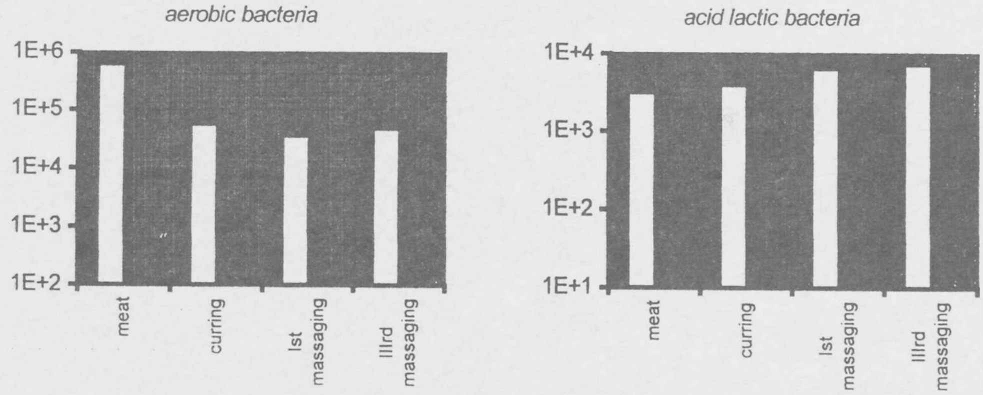

Fig.1. Effect of ultrasound on aerobic and acid lacid bacteria in pig meat (□ – control sample)

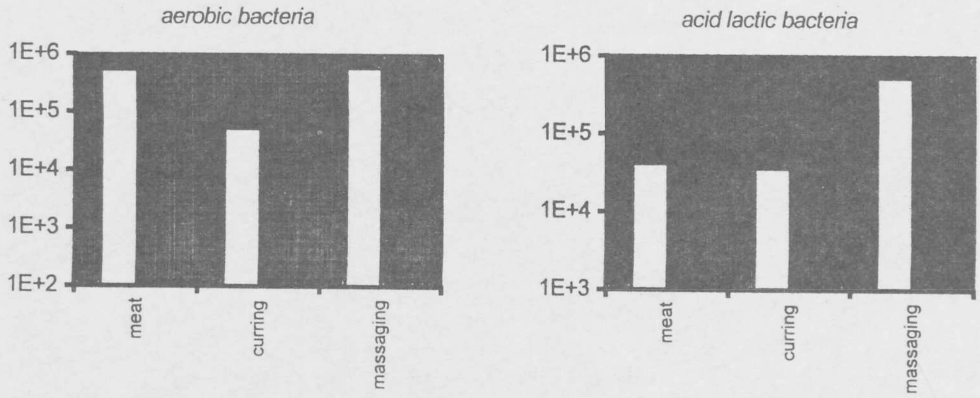

Fig.2. Effect of ultrasound on aerobic and acid lacid bacteria in beef (□ – control sample)

Collected data did not show statistically significant ultrasonically generated decreasing number of aerobic bacteria in cooked ham. However, its concentration in ultrasonicated sample was insignificantly lower compared with control sample. The total aerobic bacteria number in sliced control ham changed from $2.5 \cdot 10^2$ at the processing day up to $8.0 \cdot 10^7$ after 20 day of storage, and in ultrasonically treated ham from $1.0 \cdot 10^2$ to $7.8 \cdot 10^7$ at the last day of storage (Table 1).

Tab.1. Effect of ultrasound on aerobic bacteria of sliced ham

Cold storage [day]	Number of aerobic bacteria	
	control sample	ultrasonicated sample
0	$2.5 \cdot 10^2$	$1.0 \cdot 10^2$
3	$3.0 \cdot 10^3$	$2.1 \cdot 10^3$
6	$5.0 \cdot 10^4$	$4.2 \cdot 10^4$
10	$5.5 \cdot 10^4$	$5.4 \cdot 10^4$
20	$8.0 \cdot 10^7$	$7.8 \cdot 10^7$

Therefore, the lower total number of bacteria in meat processed with ultrasound (compared with control sample) results from some non-linear physical phenomena, such as ultrasonic cavitation. The total growth of temperature of meat during massaging and ultrasonication is lower than 1-3°C. There are no statistically significant factors of bacteria number changes. In conclusion, it can be supposed, the low-frequency and medium acoustic power ultrasound decreases the concentration of aerobic and lactic acid bacteria in processed meat. However, the effect of meat ultrasonication decays during its further processing (curing, smoking, cooking).

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